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Short Communication

Cytotoxic Effect of Eugenol on The Expression of Molecular and Osteogenic Differentiation of Human Dental Pulp Cells

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The cytotoxic effect of eugenol on the expression of molecular markers related to the osteogenic differentiation of human dental pulp cells such as collagen synthesis and the expression of two osteogenesis-related genes, alkaline phosphatase (ALP) and bone sialoprotein (BSP), was studied using human dental pulp cells (D824 cells). Cellular growth and survival were decreased by treatment of cells with eugenol in a concentration-dependent manner. The incorporation rate of [3H] proline into the acid-insoluble fraction and the synthesis of type I–V collagens were also reduced by treatment of cells with eugenol in a concentration-dependent fashion. The mRNA expression of ALP was scarcely affected in cells exposed to eugenol, whereas the mRNA and protein expression of BSP was down-regulated depending on the concentrations of eugenol. The results suggest that because collagen synthesis and BSP expression play a critical role in hard tissue formation, eugenol used for endodontic treatment may give rise to cytotoxic effects to the normal function of stem cells reported to exist in human dental pulp tissue and periodontal ligament. Eugenol (4-allyl-2-methoxyphenol) is the main component of oil of cloves (Eugenia caryophyllate). It is used as a fragrance and flavoring agent, an insect attractant, and as a topical antiseptic and anti-inflammatory analgesic in dentistry. Mixed with zinc oxide into a thick paste, eugenol is also used in dentistry as a component of periodontal dressings, impression materials, and endodontic medications. Some of the endodontic medications administered to the teeth can reach the pulp tissue or the periodontium after penetrating the enamel and dentin or passing through apical foramens, respectively [1]. If the endodontic medications were cytotoxic, they could disturb the normal function of stem cells reported to exist in human dental pulp tissue [2] and periodontal ligament [3]. It is, therefore, important to study the cytotoxicity of chemical agents used for endodontic treatment. Despite the extensive clinical use in dentistry, eugenol is cytotoxic to several types of human cells, including dental pulp cells [4], gingival fibroblasts [5], and periodontal ligament fibroblasts [6]. The cytotoxicity of eugenol shown in almost all the studies was determined by the growth or viability of cells treated with eugenol. Few studies have been performed for determining the cytotoxic effect of eugenol on the differentiation-related phenotypes in human dental pulp cells.

In the present study, we investigated the cytotoxic effect of eugenol on the expression of molecular markers related to the osteogenic differentiation of human dental pulp cells such as collagen synthesis and the expression of two osteogenesis-related genes, alkaline phosphatase (ALP) and bone sialoprotein (BSP), in human dental pulp cells. Goldberg et al. [7] have demonstrated that the osteogenesis-related proteins including collagen, ALP, and BSP are synthesized during physiological and reparative dentinogenesis. Collagen, particularly type I, composes 90% of the dentin matrix. The collagen matrix provides not only the scaffold to promote and develop a mineralized tissue, but also an excellent natural support for non-collagenous proteins such as BSP and dentin sialoprotein [7]. ALP is an essential factor in dentin mineralization and in the formation of acellular cementum [8]. BSP is one of the non-collagenous proteins found in bone, dentin, and dental pulps [9], and considered as an early marker of differentiating osteoblasts [10] and odontoblast-like cells [11]. It binds to the specific residues of type I collagen [12] and serves as a potent nucleator of hydroxyapatite formation on the collagen fibrils [11]. Human dental pulp cells (D824 cells) derived from the dental pulp tissue obtained from a lower third molar extracted from a woman (22 years old) were grown as described previously [13]. D824 cells have the capability of forming mineralized nodules in vitro and recruiting odontoblast-like cells and dentin-like tissue in immunocompromised mice [13]. All experiments were carried out using D824 cells at 10–15 passages. Eugenol (>95% pure) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and dissolved at 200 mM in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Tokyo, Japan). The solution was diluted with culture medium to the desired concentrations and applied to D824 cells. Cell survival was determined by the colony-forming efficiency of cells treated with eugenol. Cells (500) were plated in triplicate onto 60-mm dishes and incubated overnight. The cells were treated with eugenol at varying concentrations for 24 h. Control cultures were incubated with DMSO medium. After two washings with 2 ml of fresh medium, cells were incubated for 13 days for colony formation. Cells were fixed with absolute methanol and stained with a 10% Giemsa solution. Thompson et al. [19]. have demonstrated that eugenol is metabolized to the reactive intermediate, possibly a quinone methide, in isolated rat hepatocytes and that the cytotoxic effects of eugenol in hepatocytes are dependent on the metabolism. Eugenol elicits unscheduled DNA synthesis in Syrian hamster embryo (SHE) cells in the presence of a rat liver post-mitochondrial supernatant (PMS) mixture [20]. It also induces chromosome aberrations in SHE cells, and the frequencies of chromosome aber-

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rations are enhanced when the cells are treated with eugenol in the presence of a PMS mixture [21]. In vitro exposure of eugenol to the human p53 in the presence of cytochrome P450 induces DNA damages at C and G of the 5′-ACG-3′ sequence complementary to codon 273 of the gene [22]. These results suggest that the cytotoxic and genetic effects of eugenol can be associated with oxidative DNA damage by its metabolites. D824 cells have a potential for metabolic activation, because they are a mixed-cell population composed of many types of cells. Thus, the cytotoxicities of eugenol observed in the present study may be attributable to the metabolite(s) of eugenol Eugenol obtained from the manufacturers without dilution is often applied directly to dental cavities as an antiseptic and anodyne. Because the concentration of eugenol is 6.5 M, that is ≥6,500 times the concentration used in the present study, clinically applied eugenol that penetrates into the pulp tissue may give rise to adverse effects against the normal function of pulp tissue.

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